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16. ABSTRACT

A series of neurophysiological tests was performed on Long-Evans hooded rats treated with either 2-,3- or 4-methylpyridine at dosages of 100mg/kg, approximately 1/2 the i.p. LD50. The tests contained measures of sensory function (paired pluse flash evoked potentials, pattern reversal evoked potentials, brainstem auditory evoked responses) and cerebral excitability (pentylenetetrazol (PTZ) seizures and hippocampal afterdischarges). In general, rats treated with 2- and 3-methylpyridine were more affected than those treated with 4-methylpyridine. The changes observed were in many ways similar to those seen following administration of depressant compounds: increased latency of evoked potentials and increased latency to PTZ seizures. Not all findings, however, were consistent with previously observed patterns of CNS depression.

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JOURNAL ARTICLE

Neurophysiological Consequences of Acute Exposure to Methylpyridines[1]

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Neurophysiological Consequences of Acute Exposure to Methylpyridines. DYER, R. S., BURDETTE, L. J., JANSSEN, R., AND BOYES, W. K. (1985). *Fundam. Appl. Toxicol.* **5**, 920–932. A series of neurophysiological tests was performed on Long–Evans hooded rats treated with either 2-, 3-, or 4-methylpyridine at dosages of 100 mg/kg, approximately one-half the ip LD50. The tests contained measures of sensory function (paired pulse flash evoked potentials, pattern reversal evoked potentials, and brainstem auditory evoked responses) and cerebral excitability (pentylenetetrazol seizures and hippocampal afterdischarges). In general, rats treated with 2- and 3-methylpyridine were more affected than those treated with 4-methylpyridine. The changes observed were in many ways similar to those seen following administration of depressant compounds: increased latency of evoked potentials and increased latency to PTZ seizures. Not all findings, however, were consistent with previously observed patterns of central nervous system depression.
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Methylpyridine compounds are produced in a volume which may exceed 0.5 million kg/year, and are useful as organic intermediates in the production of a variety of products, including agricultural chemicals and pharmaceuticals (USEPA, 1982a–c). While pyridine, the parent compound, is known to have central nervous system (CNS) depressant properties (Leslie *et al.*, 1973), and other substituted pyridines such as acetyl and amino pyridines have profound effects on the nervous system (e.g., Coggeshall and MacLean, 1958; Mihaly *et al.*, 1983), little is known about the neurobehavioral consequences of exposure to methylpyridines. One report suggested that oral exposure to 2-methylpyridine at dosages as low as 0.05 mg/kg/day for 6 months could produce pathological alterations in a number of brain areas (Polilei, 1969).

As a first step toward assessing the potential neurotoxicity of methylpyridine compounds, we performed a battery of neurophysiological tests upon animals exposed to a single ip injection of approximately one-half the ip LD50 value. Two tests of visual function [paired flash evoked potentials (FEPs) and pattern reversal evoked potentials (PREPs)], a test of auditory function [brainstem auditory evoked responses (BAERs)], and two tests of cerebral excitability [pentylenetetrazol (PTZ) seizures and hippocampal afterdischarges (ADs)] were performed. These tests were chosen because previous studies had found them useful in detecting and characterizing neurotoxicity (e.g., Dyer, 1985; Dyer and Boyes, 1983a; Dyer *et al.*, 1979; Janssen *et al.*, 1983).

The purpose of these studies was to determine (1) whether acute exposure to a high dosage of methylpyridines produces neurophysiological consequences; (2) whether a pattern of change existed that would suggest the nature of dysfunction; and (3) whether there were differences in the magnitude and

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TABLE 1

NUMBER OF RATS BY COMPOUND, DOSE, AND TEST

Compound	Dose	Test				
		FEP	PREP	BAER	AD	PTZ
2-Methylpyridine	100 mg/kg	24	24	24	11	16
Saline		22	22	23	14	17
3-Methylpyridine	100 mg/kg	20	17	19	10	15
Saline		21	22	21	10	15
4-Methylpyridine	100 mg/kg	22	22	22	10	13
Saline		19	20	20	9	13

pattern of alterations produced by the different methylpyridines.

METHODS

Animals. All studies were performed using adult male Long–Evans hooded rats.[2] The rats weighed 250–350 g at the time of experimentation and were housed in plastic cages with wood chip bedding. Purina lab chow (5001) and tap water were available *ad libitum.* Rats with implanted electrodes were housed individually, and others (lethality and PTZ studies) were housed in groups of two to four. The number of animals in each study is indicated in Table 1. Separate saline (vehicle) control groups were used for each compound except in the rectal temperature study.

Lethality determinations. Lethality was assessed using 12, 28, and 35 rats for 2-, 3-, and 4-methylpyridine, respectively, following ip administration of dosages ranging from 100 to 300 mg/kg. All rats in a given cage received the same ip dosage of the compound. Injections were given in a volume of 1 ml/kg. Nominal purities of 2-, 3-, and 4-methylpyridines were 98, 99, and 98%, respectively.[3] Surviving animals were observed for 7 days following injection.

Temperature. To determine whether a 100-mg/kg dosage of any of the methylpyridines produced significant alterations in rectal temperature, 24 rats were divided into four groups of six each. Rats were injected ip with either saline (vehicle) or 100 mg/kg of 2-, 3-, or 4-methylpyridine. Rats were placed in plastic restraining tubes for 6 hr during which thermistor probes (YSI 402) were inserted 10 cm into the rectum and temperature readings were obtained at 15, 30, 60, 120, and 360 min after injection. The data were analyzed by a repeated-measures ANOVA, which, when significant, was followed by Tukey's HSD test.

Pentylenetetrazol seizures. Sensitivity to pharmacologically induced seizures was assessed by administering an ip injection of 50 mg/kg PTZ, 1 hr after ip injection of either saline or 100 mg/kg of the methylpyridine compound under study. Four variables were measured after injection of PTZ by an observer blind to the animal's treatment: latency to the first myoclonic jerk, duration of the seizure (from the first myoclonic jerk to the last seizure movement), seizure severity, and latency to walk after the seizure. Seizure severity was expressed as the cumulative score of the presence (1 point) or absence (0) of the following behaviors: forelimb clonus, hindlimb clonus, forelimb tonus, hindlimb tonus, and rolling or vocalization. Observations were made following injection of PTZ until there was no further evidence of seizure (i.e., animal was walking) or for a maximum of 15 min. Rats used in the PTZ study were not used for any other purpose.

Electrophysiology. For any given methylpyridine and its associated saline control group, the four electrophysiological tests (FEP, PREP, BAER, and AD) were performed on the same animals. The tests were administered in random order beginning 1 hr after treatment, with the exception that the AD test always came last to avoid potential influences of hippocampal electrical seizures on the other tests. Total time in testing was about 45 min/rat. All tests were administered to unanesthetized rats with chronic implanted electrodes. The number of animals used for a particular analysis (see Table 1) varied because of recording problems or, in the case of AD studies, failure to exhibit an AD or inaccurate electrode placement.

Surgery. At the time of surgery, all rats weighed 250–300 g. Following anesthesia with 0.37 ml/100 g body wt, Chloropent[4] (each milliliter of Chloropent contains 42.5 mg chloral hydrate and 8.86 mg pentobarbital), rats were placed in a stereotaxic frame. Stainless-steel screws (00-

[2] Charles River Breeding Co., Wilmington, Mass.

[3] Aldrich Chemical Corp., Milwaukee, Wisc.

[4] Fort Dodge Labs, Inc., Fort Dodge, Ind.

90 × 1/16 in.) were implanted in small burr holes placed in the skull at 2 mm anterior to bregma and 2 mm left and right of the midline for ground and reference, 6 mm posterior and 4 mm lateral for FEP and PREP, and 1 mm posterior to lambda (i.e., the curve of best fit along the lambdoid suture) in the midline for BAER recording. The hippocampal electrodes were bipolar twisted nichrome wires (0.125 mm), insulated except at the tip, and separated in the vertical plane by about 0.75 mm. The electrodes were implanted with the incisor bars set 5 mm above 0. Hippocampal coordinates, 2.6 mm posterior to bregma, 2.0 mm lateral from midline, and 3.0 mm below dura for the deep tip of the pair, were selected to position the electrodes in fields CA1 and dentate gyrus of the dorsal hippocampus. The hippocampal electrodes were connected to gold pins in an Amphenol plastic receptacle. Soldered nichrome wires connected the screw electrodes to gold pins in the receptacle. Following electrode implantation, the entire assembly was encased in dental acrylic and the wound was sutured shut. Each rat was administered 100,000 units of penicillin G im and returned to its cage. A 1-week recovery period was allowed before experimentation.

Paired pulse flash evoked potential test. Each rat was placed in a small chamber with mirrors on three sides and the strobe lamp of a Grass PS22 photostimulator on the fourth (Dyer and Annau, 1977). In this situation the rat was unrestrained but received a strong stimulation while facing any direction. Strobe intensity was set at 16, which produced a 10 μsec flash with an integrated photometric energy which was 4.53×10^2 lux-sec above background as estimated by a Model 450 EG&G radiometer/photometer, Model 550-2 multiprobe detector, photometric filter, and Model 550-3 pulse integrator. Background illumination was approximately 115 lux. Each trial consisted of a pair of flashes separated by 200 msec. Trials were presented at a rate of 0.3 Hz, and each animal had 128 trials averaged to produce its FEP. During trials, which were 409.6 msec long, the EEG was amplified (×10,000) and bandpass filtered (0.8 Hz to 10 kHz) before sampling at 2500 Hz and averaging by a PDP 11/70 computer. Averaged waveforms were displayed on a Tektronix 4054 terminal, and paper copies were obtained on a Tektronix 4631 hard-copy unit. Peak latencies (P1, N1, P2, N2, P3, and N3) and peak-to-peak amplitudes (P1N1, N1P2, P2N2, N2P3, and P3N3) were obtained for each of the two waveforms present in each animal's averaged response to the paired flashes. The paired flash values were treated as repeated measures in statistical analyses. Separate MANOVAs were performed upon amplitude and latency data.

Pattern reversal evoked potential test. PREPs were recorded from rats restrained in a harness[5] and placed with their eyes 7 cm in front of the television screen of a Grass VPG visual pattern generator. Black and white bars (a square wave grating of 0.2 cycles/degree, 99% contrast, 30 cd/m^2 luminance, 69 × 74 degree field size) were displayed on the screen. The bars were reversed at a rate of 0.5 Hz, black for white, so that there was no change in overall stimulus luminance. Pattern reversals and line frequency (60 Hz) were nonsynchronized. Sampling and recording were as described above for the FEP, except that 200 trials were averaged for each rat, and the bandpass was 0.8 to 100 Hz. Peak latencies (N1, P1, N2, P2, and N3) and peak-to-peak amplitudes (N1P1, P1N2, and P2N3) were obtained from each rat's averaged waveform. Separate MANOVAs were performed upon amplitude and latency data.

Brainstem auditory evoked response test. For BAER recording the rats were placed in a chamber identical to the chamber described above for the FEP. Click stimuli of 50 μsec duration were presented at a rate of 10/sec and at a level of 90 dB maximum rms sound pressure level at the animal's ear. Evoked responses were bandpass filtered between 30 Hz and 30 kHz. A total of 512 trials of 12 msec duration were sampled at 333 kHz and averaged by a Nicolet 1170 averager. Separate multivariate analyses of variance (MANOVAs) were performed upon peak latencies (N1, P1A, P1B, N2, P2, N3, P3, N4, P4, and N6) and peak-to-peak amplitudes (N1P1A, N1P1B, N2P2, N3P3, N4P4, and P4N6). Two Bonferroni-corrected ANOVAs were performed upon central conduction time, one for the difference between P4 and P1A latencies (P4-P1A) and the other between P4 and P1B latencies (P4-P1B).

Hippocampal afterdischarge test. Testing and scoring of the AD was performed in a manner similar to that described by Dyer *et al.* (1982). Rats were placed in a 60 × 60-cm plastic chamber. Recording electrodes were connected through a cable and a relay to a Grass Model 7B polygraph with Model 7PB11J EEG amplifiers with filters set at 1 Hz and 10 kHz. During stimulation the relay was switched to a Grass S88 stimulator with two PSIU6 constant-current converters. Stimuli were biphasic square waves of 0.3 msec duration in a 2-sec train of 50-Hz pulses. Each rat received an ascending series of stimulus intensities. Beginning at 10 μA, current in successive 1/min trains increased in 10-μA steps to 100 μA, and 20-μA steps to 200 μA until an AD was elicited. If no AD was elicited, the rat was eliminated from analysis. Threshold and duration of the AD, postictal depression duration, and rebound AD duration were measured.

Following completion of the AD testing, rats were anesthetized with Chloropent and perfused through the heart with saline, followed by 10% buffered Formalin. To help localize the deep electrode tips, potassium ferrocyanide was added to the Formalin solution, and anodal current of 2 mA was run through each hippocampal electrode tip for 5 sec (Prussian blue test). Frozen sections (90 μm) were obtained and stained with hematoxylin and eosin.

Data analysis. For each electrophysiological test, data were analyzed using general linear models MANOVA procedures available in SAS (SAS Institute Inc., 1979).

[5] Alice King Chatham Medical Arts, Los Angeles, Calif.

Since the same animals were used for all electrophysiology tests, the α was split among tests to maintain an overall 0.05 level. Significant MANOVAs were followed by Bonferroni-corrected ANOVAs (see Table 2).

RESULTS

Lethality determinations. As represented graphically in Fig. 1, the approximate 7-day ip LD50s for 2-, 3-, and 4-methylpyridine were 200, 150, and 162.5 mg/kg, respectively. In no case was lethality observed at 100 mg/kg, so this value was chosen for subsequent studies. Observation of treated animals suggested that the methylpyridines produced effects with a rapid onset. Animals typically became quiescent within minutes, and remained so for several hours. Lethality occurred most often 24–48 hr after treatment. Some animals dosed with 4-methylpyridine at 100 mg/kg engaged in what appeared to be a boxing posture. Little sparring was observed, but vocalizations accompanied the boxing posture for periods up to 18 hr after treatment.

Temperature. All of the methylpyridines appeared to produce a mild (0.5 to 0.75°C) hypothermia. The only compound for which the difference from control values reached statistical significance, however, was 3-methylpyridine. There was no significant dose × time interaction, suggesting that the magnitude of 3-methylpyridine-induced hypothermia did not change during the 6 hr in which data were collected. These results are summarized in Fig. 2.

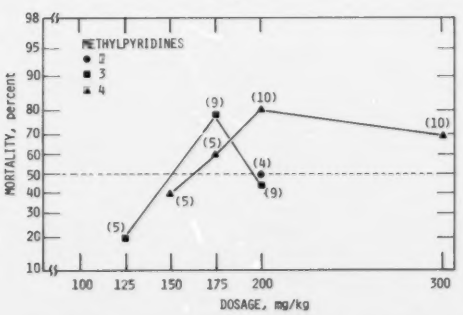

FIG. 1. Lethality data for 2-, 3-, and 4-methylpyridine. Numbers in parentheses near data points are the numbers of animals treated at that dosage.

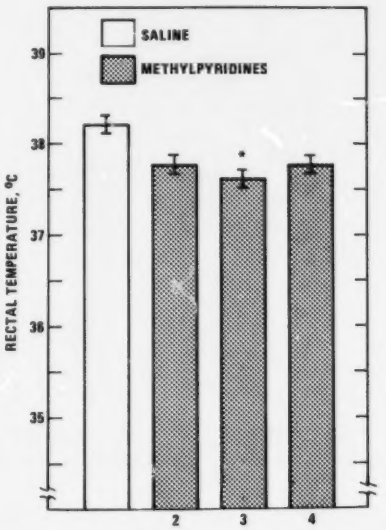

FIG. 2. Effects of 2-, 3-, and 4-methylpyridine upon rectal temperature. Values plotted are $\bar{x} \pm$ SE. Data were collapsed across the 6 hr of recording since analysis showed no treatment by time interaction. $^*p < 0.05$ (Tukey's HSD test). $n = 6$/group.

PTZ seizures. The MANOVA testing the effects of treatment upon PTZ seizures was statistically significant for 2-methylpyridine and 3-methylpyridine but not for 4-methylpyridine. Stepdown ANOVAs conducted on individual variables indicated that 2-methylpyridine exposure significantly increased latency to myoclonic jerk and decreased seizure severity. Stepdown ANOVAs conducted on the 3-methylpyridine variables showed that none of them alone reached the required level for statistical significance. These findings suggest that in this case a linear combination of effects on different variables, probably decreased latency to myoclonic jerk and decreased seizure severity (see Fig. 3), accounted for the significant MANOVA. The seizure severity and latency to myoclonic jerk data are summarized in Fig. 3, which shows that all three test compounds produced a similar pat-

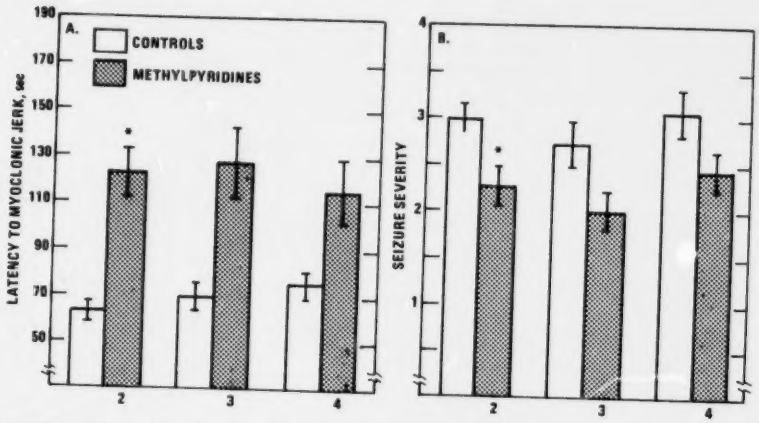

FIG. 3. Effects of 2-, 3-, and 4-methylpyridine upon (A) latency to PTZ-induced myoclonic jerks, and (B) PTZ seizure severity. Values plotted are $\bar{x} \pm$ SE.

tern of changes, but that the magnitude of these changes was slightly greater and the variability slightly less with 2-methylpyridine.

Electrophysiology tests. Bonferroni corrections were applied to the MANOVAs performed upon data acquired during the electrophysiological tests. The required (for statistical significance) and obtained MANOVA p values for each test are summarized in Table 2.

FEP test. Figure 4 illustrates group-averaged waveforms for treated and control animals in each of the three studies. It is evident that none of the treatments produced a major modification in the FEP. Statistical analysis indicated that FEP amplitudes were not significantly altered by any of the compounds studied. Both 2-methylpyridine and 3-methylpyridine increased the latencies of FEP peaks (significant MANOVA, Table 2). These results, summa-

TABLE 2

REQUIRED AND OBTAINED MANOVA p VALUES FOR ELECTROPHYSIOLOGICAL TESTS

Test	Required p value[a]	2-Methylpyridine	3-Methylpyridine	4-Methylpyridine
FEP				
Amplitudes	0.00625	0.018	0.019	0.047
Latencies	0.00625	0.0024*	0.0013*	0.60
PREP				
Amplitudes	0.00625	0.76	0.19	0.015
Latencies	0.00625	0.33	0.47	0.49
BAER				
Amplitudes	0.0042	0.0004*	0.0038*	0.0010*
Latencies	0.0042	0.0001*	0.0053	0.073
Central cnd.	0.0042	0.0001*	0.0005*	0.73
AD	0.0125	0.089	0.078	0.047

[a] Required p values were derived from α splitting to maintain overall α at 0.05.
* Achieved required p value for significance.

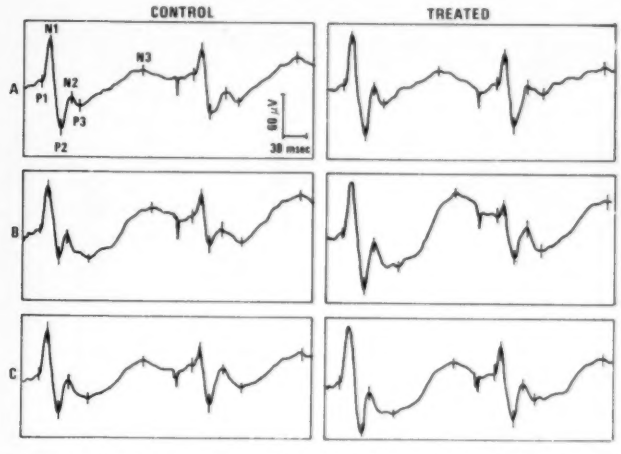

FIG. 4. Group-averaged FEP waveforms for all treated and control animals in each of the three studies. The figure shows the importance of independent control groups for each FEP study by illustrating subtle population sampling differences between studies. (A) 2-methylpyridine, (B) 3-methylpyridine, and (C) 4-methylpyridine.

rized in Fig. 5, indicate that while most peaks appeared to increase in latency after treatment, only P1 and N1 reached statistical significance. In no case was the treatment × flash interaction statistically significant, indicating that the magnitude of effect was equivalent in the waveforms evoked by the first and second flash of the pair.

PREP test. Figure 6 illustrates the group-averaged waveforms obtained for treated and control animals in each of three studies. As with the FEP, there were no obvious modifications made in the PREP by any of the compounds studied. Statistical analysis failed to reveal any significant effects of the compounds on amplitudes or latencies.

BAER test. Figure 7 illustrates representative single-animal average waveforms from each of the three studies (group averages were not available). Amplitudes of BAERs were increased by all three compounds (all amplitude MANOVAs significant). The pattern of increase (i.e., which peaks were increased) was not identical in all cases. Indeed, none of the stepdown ANOVAs revealed statistical significance in the case of 4-methylpyridine. Figure 8 illustrates the influence of 2-, 3-, and 4-methylpyridine upon amplitudes of N2P2, N4P4, and P4N6 peaks. While latencies of most BAER peaks appeared to be increased by 2-methylpyridine and 3-methylpyridine, only the 2-methylpyridine MANOVA was statistically significant. Figure 9 shows the latencies of several selected peaks for each of the three studies. Finally, the measures of central conduction time, P4 − P1A and P4 − P1B, were significantly increased (significant MANOVA, Table 2) by both 2-methylpyridine and 3-methylpyridine, but not by 4-methylpyridine (see Tables 2 and 3).

AD test. Inclusion of data in the AD analysis was contingent upon the animal (1) having an AD threshold below 200 μA and (2) having electrodes properly placed in the dorsal hippocampus. Consequently, the sample size is smaller in these analyses than those described above (see Table 1). None of the studies produced significant alterations in any AD parameters measured. During testing, however, it was noticed that ADs of some animals had an unusually complex spike in the initial stages of the AD. Figure 10 illustrates the AD of a

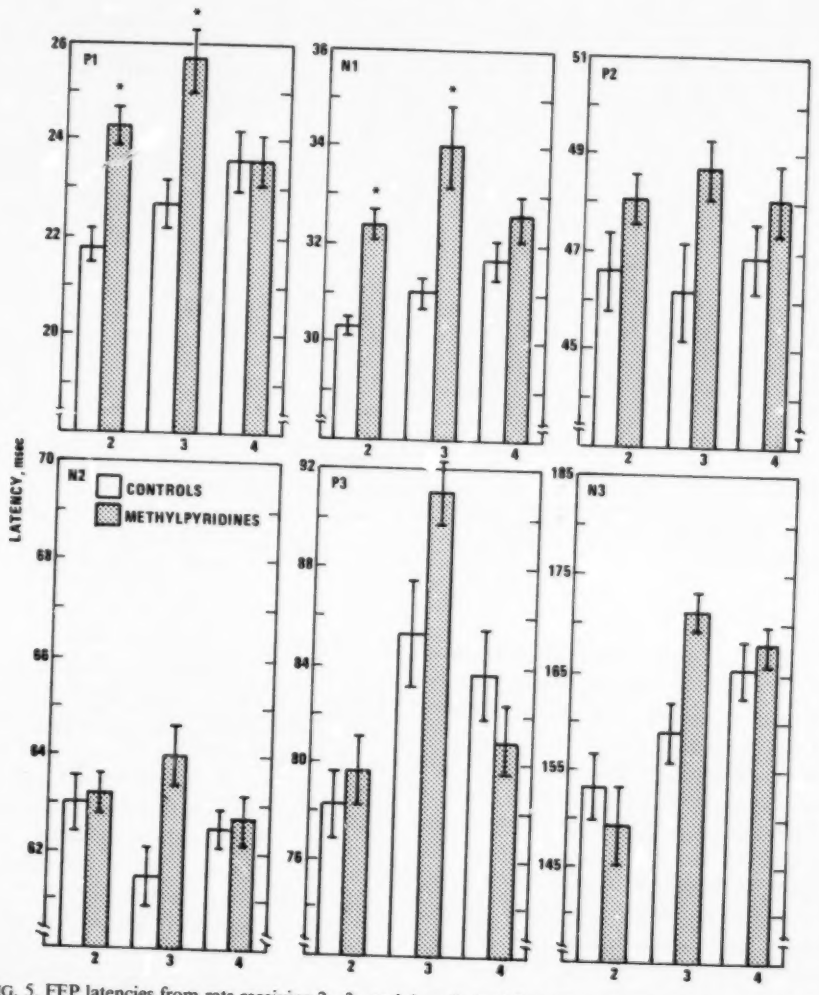

FIG. 5. FEP latencies from rats receiving 2-, 3-, and 4-methylpyridine. Since there were no dose × flash interactions, values ($\bar{x} \pm$ SE) are collapsed across flash 1 and flash 2. *Statistically significant.

control (simple spiked) and a treated (complex spikes) subject. To evaluate whether this finding was related to the treatment, all ADs were rated by two individuals blind to experimental treatment for the presence or absence of a complex spike pattern. Instances in which the two observers did not agree were discarded. A χ^2 test conducted on these ratings indicated that complex spikes were more likely to be found in rats treated with either 3-methylpyridine or 4-methylpyridine than with saline ($ps < 0.03$) (see Table 4).

DISCUSSION

These neurophysiological studies indicated that while all three methylpyridine compounds produce qualitatively similar alterations in nervous system function at sublethal dosages,

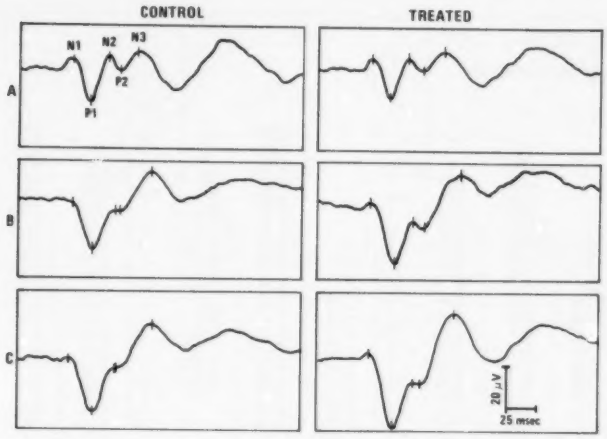

FIG. 6. Group-averaged PREP waveforms for all treated and control rats in each of the three studies. The figure shows the importance of independent control groups for each PREP study by illustrating subtle population sampling differences between studies. (A) 2-methylpyridine, (B) 3-methylpyridine, and (C) 4-methylpyridine.

2- and 3-methylpyridine were more toxic than 4-methylpyridine. In the absence of morphologically or functionally pathological data, one cannot rule out the possibility that the effects observed were secondary to toxicity in other organ systems. Most of the findings, including increased latencies of evoked potentials (FEPs and BAERs) and increased latencies to PTZ-induced myoclonic jerks, are characteristic of CNS depressant and/or anticonvulsant compounds (Dyer and Boyes, 1983b; Hetzler and Oaklay, 1981; Kasting *et al.*, 1981). No data

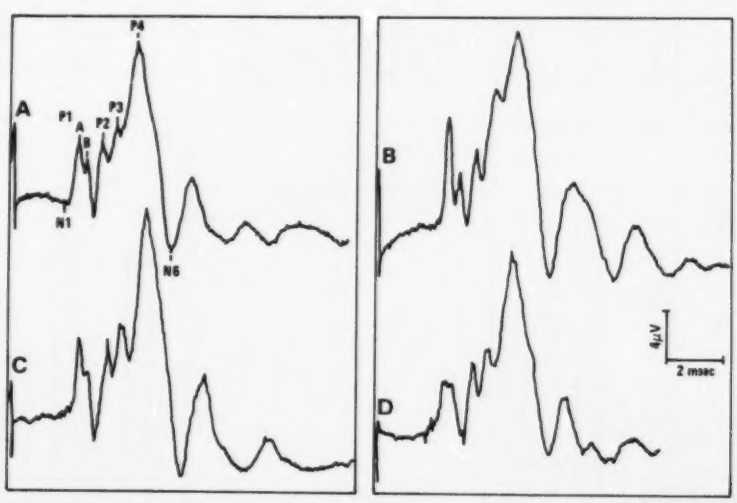

FIG. 7. Representative single-animal average BAER waveforms from each of the three studies. (A) Control, (B) 2-methylpyridine, (C) 3-methylpyridine, and (D) 4-methylpyridine.

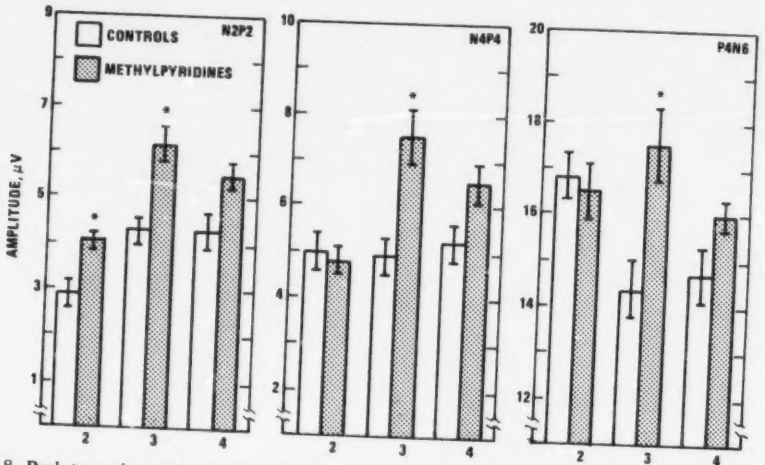

FIG. 8. Peak-to-peak amplitudes ($\bar{x} \pm$ SE) of BAER components N2P2, N4P4, and P4N6 following treatment with 2-, 3-, or 4-methylpyridine. *Statistically significant.

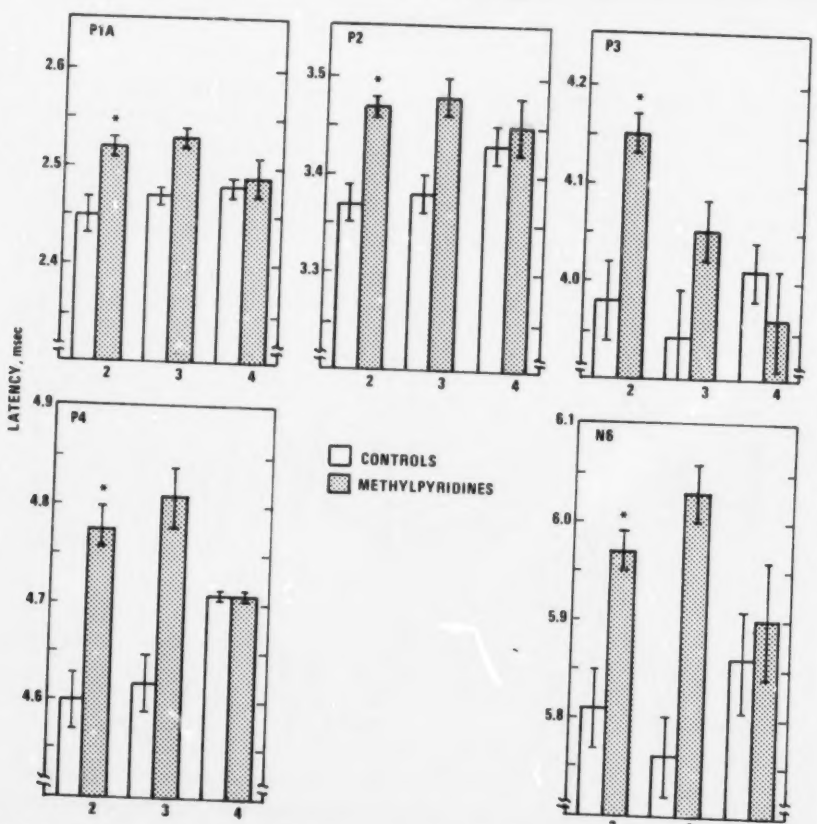

FIG. 9. Latencies ($\bar{x} \pm$ SE) from selected BAER peaks for rats treated with 2-, 3-, or 4-methylpyridine. *Statistically significant.

TABLE 3

EFFECTS OF METHYLPYRIDINES ON BAER CENTRAL CONDUCTION TIME (msec)

Compound	Dosage	P4 – P1A ($\bar{x} \pm$ SE)	P4 – P1B ($\bar{x} \pm$ SE)
2-Methylpyridine	100 mg/kg	2.26 ± 0.01*	1.87 ± 0.01*
Saline		2.14 ± 0.02	1.77 ± 0.02
3-Methylpyridine	100 mg/kg	2.28 ± 0.02*	1.97 ± 0.02*
Saline		2.15 ± 0.02	1.82 ± 0.02
4-Methylpyridine	100 mg/kg	2.23 ± 0.03	1.90 ± 0.03
Saline		2.23 ± 0.03	1.91 ± 0.03

* Statistically significant difference from saline control.

are available to evaluate whether increased BAER amplitudes also fit this pattern. However, classification of 2-methylpyridine and 3-methylpyridine as CNS depressants should not be made hastily, because there are some findings which do not fit the pattern of most known depressants. For example, many depressants [e.g. chloral hydrate (Hetzler and Dyer, 1984), and ethanol (Myers, 1981)] produce an ambient temperature-dependent hypothermia, but hypothermia was minimal with the methylpyridines (Fig. 2). In addition, depressants tend to increase AD thresholds (Dyer *et al.*, 1979; Swartzwelder *et al.*, 1979), and no significant changes were seen with methylpyridines. Finally, the pattern of change in the FEP latencies does not correspond to either an anesthetic-type depression or a hypothermia-related depression, where the magnitude of latency shift depends upon the peak, with later peaks showing greater increases in latency than early peaks (Dyer and Boyes; 1983b; Hetzler and Dyer, 1984). Instead, the pattern of effect on the FEP is more similar to what is seen following acute exposure to triethyltin when hypothermia is prevented, an increase in latency which has the same magnitude for P1, N1, and P2 (Dyer and Howell, 1982). The latter findings were interpreted as indicative of conduction velocity slowing in the absence of hypothermia, an interpretation which may apply here as well.

The BAER has been shown to reflect activity in auditory pathways (Buchwald and Huang, 1975; Starr and Hamilton, 1976; Stockard and Rossiter, 1977). Therefore, toxicant-induced disturbances in auditory function through the brainstem should be reflected by corresponding change in the BAER. The BAER detects noise-induced hearing loss in

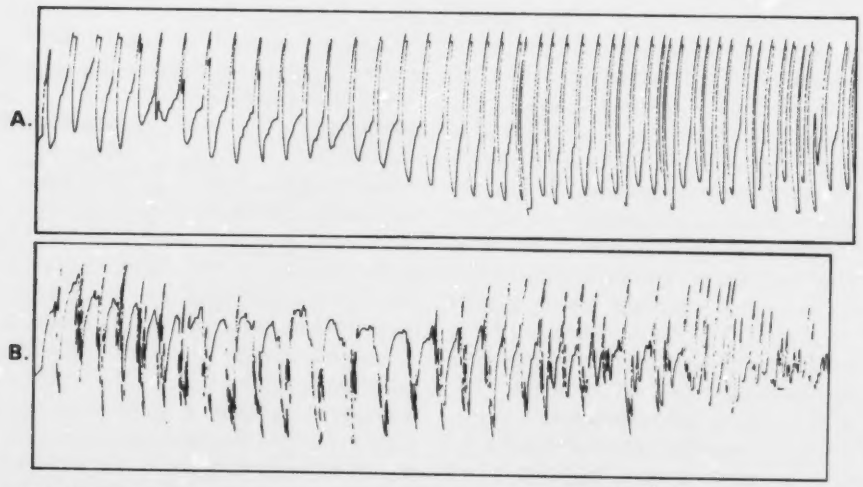

FIG. 10. Representative segments of ADs illustrating (A) a simple spike pattern and (B) a complex spike pattern.

TABLE 4

INCIDENCE OF AD SPIKE ACTIVITY BY TYPE

Compound	Dose	Simple spikes	Complex spikes
2-Methylpyridine	100 mg/kg	6/11	2/11
Saline		8/14	5/14
3-Methylpyridine	100 mg/kg	2/10	7/10
Saline		7/10	2/10
4-Methylpyridine	100 mg/kg	3/10	6/10
Saline		5/9	0/9

rats and chinchillas as effectively as the more time consuming behavioral methods (Borg, 1982; Henderson *et al.*, 1983). In toxicology, BAERs have been used to characterize peripheral hearing loss due to toluene exposure (Pryor *et al.*, 1984). Decreased BAER amplitude (often determined qualitatively) is taken as an indicator of auditory dysfunction. Increased waveform amplitude has not been reported in conjunction with peripheral damage. However, in other sensory systems, increased waveform amplitude is often associated with damage to brain areas which are both distant to those under study and not part of the primary sensory system being tested (e.g., Obeso *et al.*, 1980). Increased latency implies slowed conduction, a phenomenon which can occur for a variety of reasons, including sensory cell loss, demyelination, hypothermia, and edema. Both 2-methylpyridine and 3-methylpyridine tended to increase BAER latencies peripherally and centrally (only significant for 2-methylpyridine). Since the first positive peaks (P1A and P1B) are presumed to reflect activity in the peripheral auditory system, and later peaks activity in the brainstem, the finding that P4 − P1A and P4 − P1B were increased by 2- and 3-methylpyridine demonstrates that these compounds slowed conduction in the CNS, as well as in the peripheral auditory system.

The most sensitive electrophysiological measures in these studies appeared to be BAER amplitudes, since treatment effects were identified with all of the methylpyridines. Previous reports of alterations in BAER amplitude have described treatment induced amplitude decreases in animals with severely compromised auditory function (e.g., Amochaev *et al.*, 1979; Paravicini *et al.*, 1982). The muscarinic agonist oxytremorine has been reported to increase BAER amplitudes in the absence of latency changes (Bhargava *et al.*, 1978). Increased N4P4 amplitude has been observed in rats treated with the pesticide chlordimeform (Janssen *et al.*, 1983), and reducing body temperature by 4–5°C has produced increased amplitude of all BAER peaks in anesthetized cats (Rossi and Britt, 1984).

On the surface, the present findings of increased BAER amplitudes and increased BAER latencies produced by methylpyridines might seem to reflect hypothermia. However, this explanation is not satisfactory for several reasons. First, the pattern of latency changes observed as a function of methylpyridine exposure is different from what would be expected if changes were produced by hypothermia. Body temperatures recorded in rats treated with 2-methylpyridine and 4-methylpyridine were nearly identical (Fig. 2), yet only 2-methylpyridine significantly increased latency. Second, the magnitude of latency increase produced by 2-methylpyridine was greater than would be expected by a 0.5°C decrease in temperature. Finally, the magnitude of amplitude increase was greater than would be expected by such a mild hypothermia. For example, based on recent observations (R. Janssen *et al.*, in preparation), we estimated that a 2.2°C decrease in body temperature would have been required to produce the 3-methylpyridine N4P4 amplitude shift, and a decrease in body temperature greater than 2.5°C would have been required to produce the 2-methylpyridine P1A latency shift.

Analysis of ADs reveals the integrity of a neuronal network requiring the synchronous interplay of excitatory and inhibitory activities within and between neurons, and as such is a model for brain function in general (Dyer *et al.*, 1979). Abnormal ADs indicate disruption in the timing or balance of these activities, and

suggest disordered brain function. Hippocampal ADs were used here because they are elicited easily and manifest properties which are readily quantified (Dyer *et al.*, 1979).

The finding that rats treated with 3- and 4-methylpyridines have a higher probability of exhibiting ADs with complex spike patterns than saline-treated controls deserves further study. Complex spike ADs have been reported following high dosages of ethanol, but rats so treated also have elevated AD thresholds (Swartzwelder *et al.*, 1979), and no significant elevation of AD thresholds was produced by methylpyridines. Intense electrical stimuli can produce double or triple spike patterns in about 30% of dentate gyrus granule cells (Fricke and Prince, 1984), and the complex spikes observed during ADs may reflect such double spiking. Finally, the AD spikes represent the integrated activity of synchronously firing neurons, and disruption of the synchrony might produce a complex spike pattern. Thus, any combination of lowering the double spike threshold, changing some cells from single spike to multiple spike types, or altering the synchrony of neurons might have produced the results described here. The consequences of these findings for the functioning organism remain to be determined.

These studies provide information about the tests used, as well as about the compounds studied. For example, the different pattern of changes among tests suggests that the tests were not redundant. Of particular interest is the absence of significant alterations produced in the PREP by any of these compounds. Previous studies have shown that, depending upon the compound, the PREP may detect visual dysfunction more readily than the FEP (Boyes and Dyer, 1983). In humans the PREP may be more useful in the diagnosis of multiple sclerosis than the BAER (Purves *et al.*, 1981). Thus, each test seems useful in different situations. With regard to methylpyridines, future studies might well focus on the auditory system and measures of CNS excitability.

Finally, it must be emphasized that the intent of the present study was simply to answer whether neurobehavioral alterations could be observed following high dosages of methylpyridine compounds. The answer to this question was affirmative. All three compounds produced similar types of dysfunction which varied primarily in magnitude. It must remain for further studies to determine the time course, dose–response relationships and significance of the observed alterations.

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